

Genotypes that produced high, medium, and low numbers of embryos were selected for RNA extraction. Gene expression assays, such as DNA arrays, Northern blots, slot blots, etc., were used in attempt to correlate embryo performance with mRNA abundance for selected genes. In the example shown in Figure 9 and Table 5, expression of loblolly pine genes, designated as *Lec*, *Fie*, and *Pkl*, obtained from the Pine Gene Discovery Project, was evaluated. The preliminary correlation appears to be that the high levels of the *Lec* gene's mRNA correlates with greater number of pine embryos. (See table 5.) These experiments can be further expanded to incorporate additional or alternative genotypes with the prospect of identifying a large collection of gene indicators of good or poor performance in tissue culture based on high or low mRNA levels. It is clear from the above that this approach, using the sequences disclosed in this application, can evaluate a genotype entering tissue culture, saving both time and expense.

Somatic Embryos

[0177] Immature zygotic seeds were collected from loblolly pine genotype 260 (mother tree BC-3, Boise Cascade). Somatic embryos were initiated as described by Becwar et al. (1990) or with modifications in media mineral composition. The early stage somatic embryos were grown in cell suspension culture medium 16 and subcultured every week (Pullman and Webb, 1994). The embryos collected from the suspension, which include stage 1 and stage 2 somatic embryos, are referred to as stage S embryos. At the end of the subculture week, the somatic embryos in the suspension were settled in a cylinder and transferred to maturation medium 240

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(Pullman and Webb, 1994). Resulting somatic embryos were selected, staged, sorted into vials containing the same stage, and stored at -70°C until analyses were performed.

Probes

[0178] For the following example analysis RNA was isolated from embryos at different stages in development, early stage somatic embryos and late-stage somatic embryos. The cDNA probes used in this example are not contained in the SEQ ID NOS: 1-327, but rather, are generic, publicly available pine sequences obtained from the Pine Gene Discovery project located at

(<http://www.cbc.med.umn.edu/ResearchProjects/Pine/DOE.pine/index.html>). These clones are homologs to the well-studied *Arabidopsis* genes that have been shown to have significant influence on embryo development in this plant. The pine clone names (first column) and corresponding references for the *Arabidopsis* homologs are shown in Table 4. The three clones listed, *Lec*, *Lie*, and *Pkl*, are for representative purposes within this example and it will be clear to one skilled in the art that any of the SEQ ID NOS: 1-327 could be substituted for those here as all will help identify conditions for improved performance in culture.

[0179] Probes were made by preparation of DNA using Wizard Minipreps (Promega, Madison, WI) and cDNA inserts isolated by restriction enzyme digestion. For the cDNA probes, 50 ng of the isolated cDNA insert DNA was used to make ³²P-labeled probes with Ready-To-Go DNA labeling beads (Amersham Pharmacia Biotech) according to manufacturer's instructions. Blots were prehybridized (7% SDS, 1% BSA, 0.25 M NaPO₄ (pH 7.2), 1.0 mM EDTA) for 3 hours at 65°C and hybridized in fresh buffer at 65°C for 12 to 18 hours (4). Each blot was washed 6 times with the following

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conditions: 1) RT, 2X SSC, 0.1% SDS, 15 min; 2) RT, 2X SSC, 0.1% SDS, 30 min; 3) 42°C, 0.2X SSC, 0.1% SDS, 15 min; 4) 42°C, 0.2X SSC, 0.1% SDS, 30 min; 5) 60°C, 0.2X SSC, 0.1% SDS, 30 min; 6) 60°C, 0.2X SSC, 0.1% SDS, 30 min. Blots were exposed to a phosphorimaging plate for 10 minutes. Screens were read with a BAS1800 (software v1.0) and images were manipulated with ImageGauge (v2.54) (Fuji Photo Film Co., Ltd., Kanagawa, Japan).

[0180] The hypothesis tested within this example is that genotypes that produce large numbers of embryos have high *Lec* expression and low *Pkl* expression, poor genotypes have the opposite pattern, and that *Lec* and *Pkl* expression act as indicators of embryogenic potential. Figure 9 shows that *Lec* is not expressed in late stages of embryogenesis in somatic embryos. The *Lec* gene is expressed throughout embryogenesis in *Arabidopsis*. The blot reveals that the *Lec* gene is a useful early expression marker for embryogenesis. One interpretation of these results is that the somatic embryos do not express *Lec* in the manner that *Lec* is expressed in zygotic embryos, i.e. the use of *Lec* expression has highlighted a defect in gene expression in somatic embryos. This defect could be used to identify desirable genotypes, i.e. those likely to progress through development and produce a large number of healthy plantlets compared to undesirable genotypes that will cease development prematurely or produce low numbers of plantlets. This is an example of the principle described pictorially in Figure 8.

[0181] The results described in the previous section of Example 5 reveal ways in which gene expression analyses can be used to improve somatic embryogenesis based on several genes. However, this principle applies as well when the assay is

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